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Hemoglobin magnetism in aqueous solution probed by muon spin relaxation and future applications to brain research

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Abstract: A marked difference in spin relaxation behavior due to hemoglobin magnetism was found for positive muons (μ^+) in deoxyhemoglobin in comparison with that observed in oxyhemoglobin in aqueous solution at room temperature under zero and external longitudinal magnetic fields upto 0.4 Tesla. At the same time, small but significant unique relaxation pattern was observed in nonmagnetic oxyhemoglobin. Combined with our previous measurements on hemoglobin in human blood, application of this type of measurement to the studies of the level of oxygenation in various regions of the human brain is suggested.

Keywords: Muon, muon spin relaxation, hemoglobin, blood magnetism, oxygenation

Introduction

Because of the large magnetic moment of the μ^+ (3.2 times that of protons) and unique time window for the dynamics of the surrounding local fields, the μ^+ can easily detect a static and dynamical mG field in the atomic-level microscopic space. Also, because of the muon polarization phenomena of particle-physics-laws, the muon spin relaxation (μ SR) method can be applied to any substances at any temperatures under zero external magnetic field. This feature provides a strong advantage for μ SR over NMR, ESR, etc.¹⁾

Throughout the major part of the present study of hemoglobin in aqueous solution, our focus will be on the nature of its characteristics as a probe of weak magnetism, although it is now known that the muon

can probe not only weak magnetism but also electron conductivity through the labeled electron method.²⁾

It should be emphasized that the possible location of μ^+ in a large macromolecule containing a heme-Fe protein might be favorable for us to detect magnetism within the Fe-core; μ^+ is preferentially situated at negatively charged part near the positively charged Fe, as evidenced in a series of the experiments on electron-transfer proteins.^{1), 2)}

Now, let us summarize the well-known facts of the magnetic properties of blood. Hemoglobin (Hb) is known to be the major component of the red blood cells (which are $\sim 40\%$ by volume of whole blood), namely, Hb is about 15% by weight of whole blood. It occurs in three types; 1) oxyhemoglobin with Fe(II) (non-magnetic), 2) deoxyhemoglobin with Fe(II) (magnetic) and 3) methemoglobin with Fe(III) (magnetic).³⁾ The covalent bonding of unpaired electrons in both Fe and oxygen makes oxyhemoglobin nonmagnetic. In the lung of human body, oxygen is absorbed, forming oxyhemoglobin and in muscles and other tissues, by releasing oxygen, deoxyhemoglobin is formed. In the human body, formation of methemoglobin is suppressed by the action of enzymes. Similar processes are known to take place within the brain, where metabolism related to active function requires oxygen and influences the extent of oxygenation of hemoglobin providing an indirect signal related to brain function. Thus detecting hemoglobin magnetism is a potential basis for brain function studies such as now obtained by

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functional MRI.⁴⁾

Due to the sensitive nature of μ SR as a magnetic probe, it is expected to detect the magnetism of hemoglobin and identify the type of hemoglobin. Once it is established, along with the development of an advanced muon beam as described later, muons will be able to provide us with a measure of the oxygenated status of the blood in a small region (below a few mm)³⁾ and deep in the body (deeper than 10 cm) for the first time. Thus, this important information will become available for the future biomedical application, including study of brain function.

Experiment

At both Muon Science Laboratory of the High Energy Accelerator Research Organization (KEK-MSL) in Japan and TRIUMF in Canada, we have conducted muon spin relaxation studies on the polymerized hemoglobin of bovine origin in a lactated Ringer's solution at 13% concentration. This preparation is commercially available as OXYGLO₂BIN from BIOPURE Co. Ltd. The original sample, which is violet-colored, was taken as deoxyhemoglobin. This solution was able to be changed by supplying wet oxygen gas over the surface into oxyhemoglobin which is red-colored. The oxyhemoglobin was then reconverted to deoxyhemoglobin (violet colored) by flowing wet nitrogen gas over the solution. The characterization of each of the two forms was confirmed by light absorption spectroscopy. Almost 100% concentration of each component was always confirmed, after 10 min for deoxy-Hb to oxy-Hb transformation and after 30 min for oxy-Hb to deoxy-Hb transformation. The analysis was made repeatedly before and after each run of the muon experiments.

The 10 MeV pulsed polarized positive muon beam from the superconducting muon channel at KEK-MSL was stopped in two types of the samples, all contained in a thin plastic bag. As described earlier, the samples we used were the thus prepared samples of deoxyhemoglobin aqueous solution and oxyhemoglobin aqueous solution. The muon experiment was conducted at room temperature under zero and longitudinal external magnetic field upto 0.4 Tesla by using Superconducting Longitudinal Coil set-up. Due to the finite time width of the pulsed muon beam, it is difficult to measure relaxation phenomena in a time range below 100 ns. Previously, similar measurements had

been conducted for oxyhemoglobin-containing human blood and deoxyhemoglobin-containing human blood, where a characteristic difference in muon spin relaxation due to hemoglobin magnetism was detected.⁵⁾

The same type of measurements was conducted by using the continuous muon beam at M9B channel, TRIUMF in order to observe the muon spin relaxation phenomena at earlier time range (less than 100 ns).

Among the obtained sets of data, typical time-spectrum data at zero-field and at room temperature are shown in Fig. 1a and 1b. There, the average polarization of the muon stopping in the whole sample is presented as a function of time after muon-stopping. The absolute value of polarization was obtained by the saturated value of the positron asymmetry in the decoupling measurement (the polarization recovery measurement under the magnetic field applied along the initial muon polarization direction) shown later for the 100% value and the amplitude of the muon spin rotation in *Al* for the baseline (0% asymmetry) determination. A clear difference between μ^+ in deoxy-Hb versus μ^+ in oxy-Hb in aqueous solution was seen in earlier time range at 100 ns; the asymmetry at 100 ns being 82% versus 68% (Fig. 1a) and a weak but significant difference in spin relaxation rate in the time region from 1 to 10 μ s (Fig. 1a). The enhanced reduction of the muon polarization in the earlier time-range in the deoxy-Hb sample should be related to a muon spin depolarization by a fluctuating local magnetic field from paramagnetic heme-Fe magnetic moment.

This picture was supported by the spin relaxation data under both weak and strong longitudinal decoupling magnetic fields whose results are seen in Fig. 2a and 2b. There, data consistent with the longitudinal-field decoupling of the fluctuating field were obtained: an earlier-time-range relaxation in terms of both asymmetry and relaxation rate was significantly recovered by a small longitudinal field of 20 G in oxyhemoglobin, while the asymmetry is not affected and the relaxation rate only is weakly recovered in deoxy-Hb (Fig. 2a); a significant reduction of the asymmetry at 1 μ s exists for the deoxy-Hb at 1 kG region (83%) which is restored to the value of the oxy-Hb (95%) only above the 3 kG decoupling field (Fig. 2b).

After analyzing the obtained data, we came to the conclusion that there should be two types of the

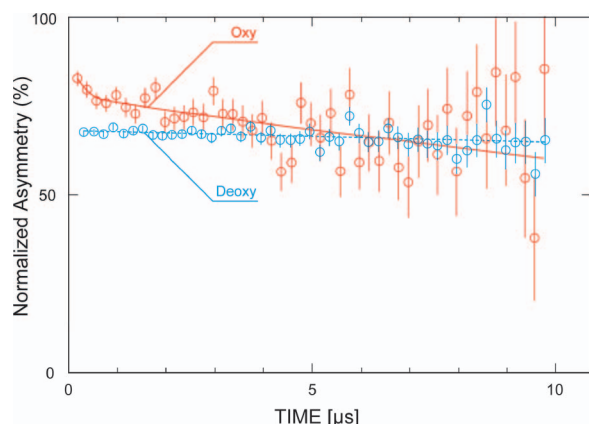


Fig. 1a. Muon spin relaxation time spectrum in hemoglobin aqueous solution at room temperature in a long time range observed with pulsed muon beam at KEK. The data is presented in terms of muon polarization as shown in the text.

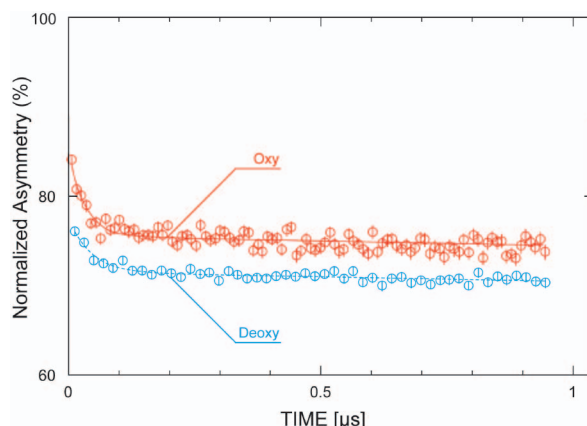


Fig. 1b. Muon spin relaxation time spectrum in hemoglobin aqueous solution at room temperature in an earlier time range observed with continuous muon beam at TRIUMF. The data is presented in terms of muon polarization as shown in the text.

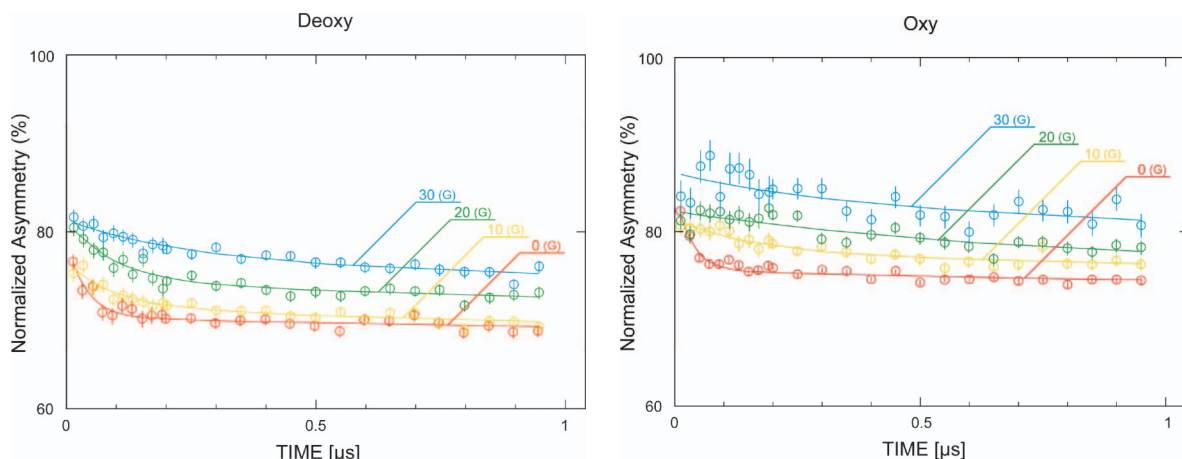


Fig. 2a. Muon spin relaxation time spectrum for positive muon in deoxyhemoglobin and oxyhemoglobin in aqueous solution at room temperature under up to 30 G longitudinal field. It is clearly seen that the earlier-time relaxation is maintained in deoxy-Hb and it is suppressed in oxy-Hb.

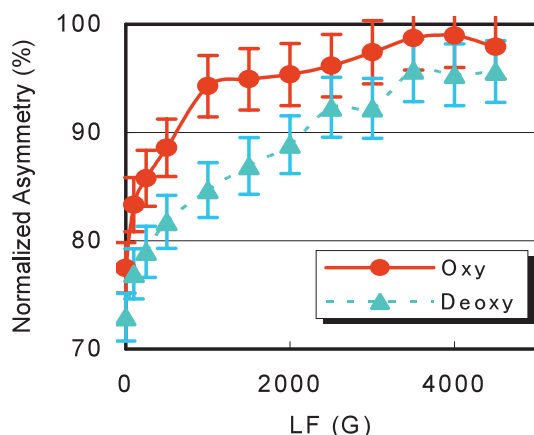


Fig. 2b. Longitudinal-field decoupling pattern representing asymmetry at around 1 μ s versus applied longitudinal magnetic field for positive muon in oxyhemoglobin aqueous solution and deoxyhemoglobin solution at room temperature.

fluctuating local magnetic field at the muon site in deoxy-Hb: 1) a weak fluctuating field exhibiting in the earlier time relaxation-recovery phenomena under a weak magnetic field (left of Fig. 2a), where, after analyzing the data through Redfield theory,^{6a)} the fluctuating field of 25 G for μ^+ (25 mG for Muonium) and a correlation time of 3×10^{-6} s is suggested; 2) a stronger fluctuating field to explain the decoupling pattern seen in Fig. 2b, where, without dynamics, the decoupling pattern can only be explained by the existence of tightly bound muonium (tighter than a bare muonium in vacuum) so that an existence of the muonium-like state which is subject to the fluctuating paramagnetic Fe moment should be considered^{6b)}.

On the other hand, in oxy-Hb, there is a long-

time relaxation seen in Fig. 1a. Both experimental magnetic susceptibility measurements^{7a)} and theoretical investigations^{7a)} have suggested a triplet spin-state of oxyhemoglobin at an excitation energy of 216 K. Consequently, there will be a significant population at room temperature in the magnetic triplet state leading muon to experience spin-lattice relaxation effect. At the same time, in order to explain the observed decoupling patterns of both Fig. 2a and 2b, an existence of weakly bound muonium which is subject to non-magnetic Fe spin can be suggested.

In order to explain all of these observations, although there should be further experimental studies as summarized later, one possible picture can be considered as follows. A neutral muonium state is formed around μ^+ . It is subject to the fluctuating Fe spin in deoxy-Hb where the double relaxation through fluctuating nuclear magnetism in surrounding atoms near Fe spin⁸⁾ causes the origin of the low-field part of the decoupling data (left of Fig. 2a) and the dynamical response of the muonium electron to the fluctuating Fe spin can explain the high-field decoupling data (Fig. 2b). At the same time, the similar muonium state placed under mostly non-magnetic Fe spin can explain the data for oxy-Hb.

The temperature dependence of the muon spin relaxation phenomena was also investigated for deoxy-Hb case. A significant change was not detected against a temperature change of 10 degrees. On the other hand, in view of the possible importance of the low-lying triplet state in oxy-Hb, it would be interesting to study temperature dependence of muon spin relaxation rate for oxy-Hb.

Discussion and future direction towards brain function studies

Similar muon experiments had been conducted for the hemoglobin-containing human blood at KEK.⁵⁾ There, the blood samples used were either the one which was kindly supplied by a male student or the one purchased from a company. It was further oxygenated by flowing wet oxygen gas for one hour until it became red-colored, which was used as oxyhemoglobin sample for the μ SR experiment. Then, it was deoxygenated by flowing wet nitrogen gas on the surface of the same sample for one hour until it became violet-colored, which was used for the experiment as deoxyhemoglobin sample. By optical absorption analysis, we confirmed a significant fraction of these samples took the chemical form as expected.

Almost similar results of muon spin relaxation were obtained; characteristic features of the zero-field relaxation and longitudinal-field decoupling (Fig. 3) are essentially the same as those for the hemoglobin in aqueous solution (Fig. 2b). It should be noted that the local concentration increase of Hb inside each blood cell does not affect the overall feature of the decoupling pattern. Thus, these results are consistent with the picture that the major part of muon spin relaxation in deoxy-Hb is explained by the fluctuating paramagnetic Fe spin, while that in oxy-Hb is explained by the mostly non-magnetic Fe spin.

At the same time, it should be noted that the data on oxyhemoglobin both in aqueous solution and in human blood at zero-external field shows a significant fast and slow depolarization. The data is unexpected since there is no magnetic spin in oxyhemoglobin. Some of the possible explanations should be as follows: 1) effect of the low-lying triplet state and 2) muonium is formed with the relevant values of spin exchange and chemical reaction.

Now, the μ SR was proved to be sensitive to the magnetism of hemoglobin in both aqueous solution and human blood. The muon location, local magnetic field, correlation time, etc. are in favor for the potential use of muon spectroscopy to detect hemoglobin magnetism in both water solution and human blood. In order to determine muon location and local electronic structure, more involved μ SR studies such as paramagnetic shift measurement, RF-resonance, level crossing resonance, etc. are required.

First-principles studies within the framework of density functional theory^{9a)-9d)} were carried out to determine the location of the positive muon and muonium in hemoglobin. The major concern of our investigations was to understand how the selective positioning near the heme Fe changes from deoxy-Hb to oxy-Hb due to the expected formation of a covalent bonding in oxy-Hb. The binding of a single positive muon to the four pyrrole nitrogen atoms was computationally analyzed through the cluster approach,¹⁰⁾ taking the heme unit and the five-membered ring part of the imidazole ligand into account, as well as one oxygen molecule above the iron in the case of oxyhemoglobin. Dangling bonds at the edge of the cluster have been terminated with hydrogen atoms. In all our calculations, we allowed the positions of the muon and the selected nitrogen

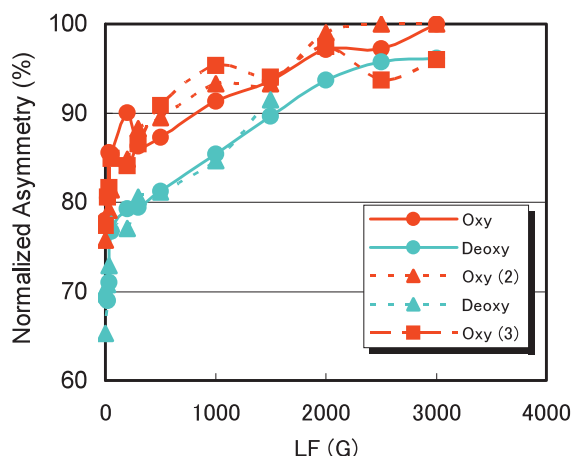


Fig. 3. Longitudinal decoupling pattern representing asymmetry at around $1\ \mu\text{s}$ versus applied longitudinal magnetic field for positive muon in oxyhemoglobin containing human blood and deoxyhemoglobin containing human blood at room temperature taken from our previous work.⁵⁾

binding partner to relax until internal forces dropped below a specific threshold.

The four pyrrole nitrogen sites are slightly inequivalent due to the presence of the imidazole ligand. Nevertheless, we observed essentially the same trends for both the N(2) site and the N(3) site; assignments of both sites are presented in Fig. 4. The muon binding energy at N(3), when facing towards the side of the heme unit where imidazole is located,^{*)} is found to be 0.32 eV higher for deoxy-Hb than for the analogous case in oxy-Hb. For the opposite orientation of muon, away from the imidazole side (see Fig. 4), the presence of the oxygen molecule affects the bonding of muon to the pyrrole nitrogen more strongly. For N(3) this leads to a binding energy of the positive muon which is 0.53 eV smaller for oxy-Hb than that for deoxy-Hb. Clearly, the competition of the oxygen molecule for the positive muon leads to a weakening of the muon-nitrogen bond. For the N(2) site, our computational study even showed that the muon can be pulled away from the nitrogen atom altogether, preferring to attach itself to the far oxygen atom of the oxygen molecule, which is posi-

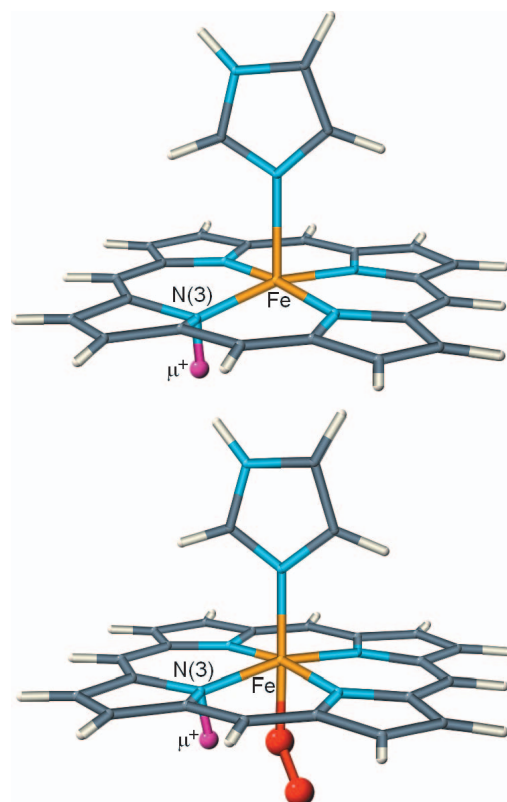


Fig. 4. Some of the results of theoretical calculations for one of the possible muon site in hemoglobin near to the Fe ion, representing at N(3) site in pyrrole ring with bonding orientation away from imidazole for the case of deoxy-Hb (upper) and oxy-Hb (lower).

tioned approximately above the center of the Fe-N(2) bond. While these results help to shed some light on the different environments encountered by the positive muon in deoxy-Hb and oxy-Hb, a more detailed analysis is required to completely understand the origin of the observed difference in the muon relaxation. A thorough analysis of the geometries for the positive muon trapping site is also needed in deoxy-Hb and oxy-Hb, comparing the results for the present DFT calculation with those from Hartree-Fock-Roothaan investigation¹⁰⁾ with explicit first-principles incorporation of many body effects.

There is another reason for us to be tempted to consider muon probe application to the humane brain. Due to the recent progress in muon beam technology, we can expect a realization of the generation of the pencil-beam like straight-line muon beam with a sectional beam-size of less than mm.^{11), 12)} The key-components are a) a large acceptance muon collection, b) the muon deceleration and/or the muon

*) A similar binding site has been theoretically observed for positive muon on the heme of cytochrome c, using Hartree-Fock-Roothaan procedure,¹⁰⁾ with the N(3)- μ^+ vector being about 1.02 Å in length and making an angle of 28 degree with the heme normal, the μ^+ being pulled towards the imidazole nitrogen, while in the present calculation in deoxy-Hb using the DFT approximation, the N(3)- μ^+ bond is oriented close to the direction of the heme normal.

cooling e.g. by a full-stopping in hot noble metal producing thermal muonium followed by ionization with e.g. lasers for a generation of the sub-eV muon-ion-source and c) muon acceleration up to MeV energy region. The precise control of muon stopping region can be made below a few mm along the beam axis (z-axis) by selecting the momentum and its width (p_μ , δp_μ) during the muon acceleration. In addition, by using position-sensitive μ SR detection system of a weak magnetism probing for the brain research, one can expect less than a few mm spatial resolution along the direction perpendicular to the detector plane.

The volume of the blood will be 3 ~ 5% of the volume of the muon stopping region, when we do not have any special selectivity of the location of the muon stopping. However, as already mentioned, we may expect enhanced localization of the stopping region of the muon near the negatively charged part next to the heme-Fe. Among the possible other heme-proteins appearing in the brain, the muon signal from hemoglobin is unique to be exhibiting a characteristic fast relaxation, while the muon in e.g. electron transferring protein takes slow relaxation (a few μ s) with a characteristic field dependence.^{1), 2)} When a part of muon stops in the rest of the brain constituents like neurons or lipids, the signal should be non-relaxing so that the signal amplitude from hemoglobin will be reduced. However a characteristic fast-relaxing nature will be enough to be used for the signal identification.

In order to consider the significance of the present μ SR method for the brain function studies, in comparison with the presently available methods, we can summarize all the methods in terms of a) principle, b) spatial resolution and depth in the brain, c) required time duration for the analysis and d) typical research objectives.

- Functional MRI; a) nuclear magnetic resonance imaging applied for the specific part of the brain, measuring resonance signals (intensity, relaxation time, etc.) under some Tesla magnetic fields, probing paramagnetism of deoxy-hemoglobin in brain blood-flow, b) a few mm resolution and deep in the brain, c) a few 10 sec, d) brain activities in response to sensory stimuli during the change of consciousness.
- PET; a) positron emission tomography from the radioactive species of e.g. oxygen externally in-

jected and reaching to the specific part of the brain as tracers in blood and its metabolite, b) 15 mm resolution and deep in the brain, c) a few min, d) brain activities during emotional responses.

- Optical topography; a) Spectroscopy of the reflected light of infrared laser probing oxidization of blood flow, b) 20 mm resolution and out of field of view, c) 0.1 sec, d) brain activities during learning and education.
- Present Proposal (Muon Spin Probes); a) detecting weak magnetism of blood by measuring spin relaxation as a function of time under zero external field, b) below a few mm resolution and any part of the brain, c) at most a few sec, d) new and complimentary information to the other methods.

As seen in the above comparison, the significance exists in the use of muons for brain function studies, which might produce a revolutionary development of brain research. Regarding human protection, the expected radiation dose was estimated. When the data is taken for $(5\text{ cm})^2$ sectional area in 100 sections, namely in a $(5\text{ mm})^3$ volume unit, the expected dose is around 0.1 mSv, which is an order of magnitude smaller than that for PET.

At the same time, as a really new type of measurement, it is interesting to investigate the possibility of the measurement of neuronal electrical activities. By using the advanced pencil-like muon beam, one can imagine an insertion of the muon magnetometer into brain non-invasively. Some complementary information can be obtained with regards to, magneto-encephalography (MEG), which is detecting magnetic flux due to neuronal electric current by SQUID spectrometer with a probing area of 15 mm and shallow part of the brain.

Conclusion

There are some subjects to be further investigated experimentally: 1) the location of the muon site with reference to the position of the Fe ion should be clarified by measuring the value of the paramagnetic shift; 2) the effect of oxygen gas used for a transformation from deoxy-Hb to oxy-Hb should be checked for the same host solution without hemoglobin, although the apparent behavior of the relaxation signal seems to be opposite, namely

more magnetic for less oxygen; 3) the origin of the high field part of the fluctuating field in deoxy-Hb must be clarified by employing the RF resonance technique in order to see spin dynamics under the applied longitudinal field; 4) the origin of the muon spin relaxation in oxy-Hb should be studied under the light of existence of low-lying triplet state.

The obtained experimental data, although more detailed experimental studies as well as theoretical understandings are required, represents the first use of μ SR for an observation of a clear difference in signal between oxy-Hb and deoxy-Hb; a reduced initial asymmetry at 0 external field and suppressed recovery of asymmetry under the decoupling external fields for the deoxy-Hb. The result is encouraging further studies and providing a nice message for a possibility of future applications of the muon spin probes to brain research.

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